

## PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Haugland and Yue )

Serial No.: 09/557,275 )

Examiner: C. Maupin )

Filed: April 24, 2000 )

Group Art Unit: 1637 )

For: **Aza-Benzazolium Containing  
Cyanine Dyes** )**CLEAN VERSION OF THE CLAIMS** )

Assistant Commissioner for Patents  
U.S. Patent and Trademark Office  
Washington, D.C. 20231

Dear Sir:

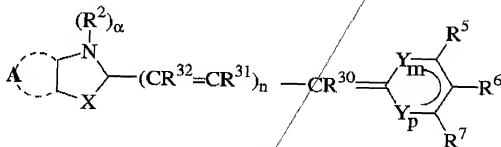
The following Clean Version of the Claims is hereby submitted together with a  
Marked-Up Version of the Claims and the Response to the Restriction Requirement on  
or before the due date of August 22, 2002.

## CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HERewith ARE  
BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADEMARK OFFICE ON 8/21/02 TO  
1.703.872.9306 By James M. Williams

Haugland and Yue  
Serial No. 09/557,275

1. (Amended) A compound having formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R<sup>2</sup>)<sup>β</sup>, provided at least one of said ring atoms is N(R<sup>2</sup>)<sup>β</sup>, wherein aromatic carbon atoms are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, -L-R<sub>x</sub> and -L-S<sub>c</sub>;

X is selected from the group consisting of O, S, Se, NR<sup>15</sup>, and CR<sup>16</sup>R<sup>17</sup> wherein R<sup>15</sup> is hydrogen or an alkyl group having 1-6 carbons and R<sup>16</sup> and R<sup>17</sup> are independently alkyl groups having 1-6 carbons, or R<sup>16</sup> and R<sup>17</sup> taken in combination complete a five or six membered saturated ring;

α is 0 or 1 and β is 0 or 1 provided that α + all β = 1;

R<sup>2</sup> is selected from the group consisting of -L-R<sub>x</sub>, -L-S<sub>c</sub>, TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by sulfo, carboxy, or amino;

n = 0, 1 or 2;

Y is -CR<sup>3</sup>=CR<sup>4</sup>;

Haugland and Yue  
Serial No. 09/557,275

p and m = 0 or 1, such that  $p + m = 1$ ;

*Sub 81*

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , TAIL, BRIDGE,  $-L-R_x$  and  $-L-S_c$  wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(CH_2)_2-W-(CH_2)_2-$  where W is a single bond,  $-O-$ ,  $-CH_2-$ , or  $-NR^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

*a1*

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of hydrogen, halogen,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , a CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$ , and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

$R^5$  is selected from the group consisting CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$ , a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of hydrogen, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$  is BRIDGE;

wherein

L and BRIDGE are independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

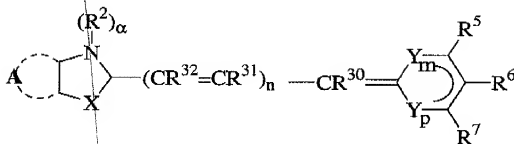
Haugland and Yue  
Serial No. 09/557,275

$R_x$  is a reactive group;

$S_c$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula

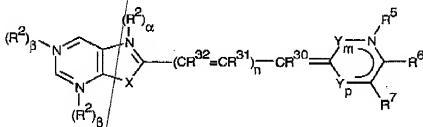
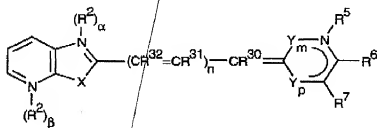
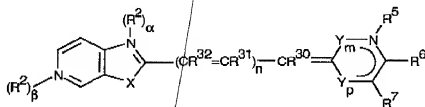
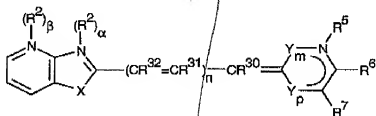


wherein A, X,  $R^2$ ,  $\alpha$ , n,  $Y_m$ ,  $Y_p$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{30}$ ,  $R^{31}$  and  $R^{32}$  are as defined above provided that BRIDGE not be any of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$ .

2. (Amended) The compound according to Claim 1, having the formula selected from the group consisting of

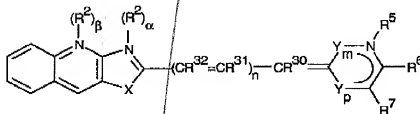
Hangland and Yue  
Serial No. 09/557,275

Sub  
B1  
a1



and

Haugland and Yue  
Serial No. 09/557,275



3. (Amended) The compound according to Claim 1, wherein said TAIL comprises formula LINK-SPACER-CAP;

wherein

LINK is a single covalent bond, -O-, -S-, or -NR<sup>20</sup>-; where R<sup>20</sup> is hydrogen, a linear or branched alkyl having 1-8 carbons, or -SPACER'-CAP';

SPACER and SPACER' are individually covalent linkages that are linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-16 nonhydrogen atoms selected from the group consisting of C, N, P, O and S;

CAP and CAP' are individually -O-R<sup>21</sup>, -S-R<sup>21</sup>, -NR<sup>21</sup>R<sup>22</sup>, or -N<sup>+</sup>R<sup>21</sup>R<sup>22</sup>R<sup>23</sup>ψ';

wherein

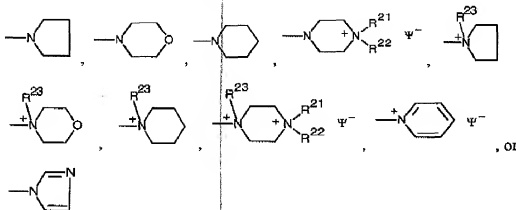
R<sup>21</sup>, R<sup>22</sup>, and R<sup>23</sup> are independently hydrogen or a linear or branched alkyl having 1-6 carbons or cycloalkyl having 3-8 carbons, wherein said alkyl is optionally further substituted by substituents selected from the group consisting of halogen, hydroxy, alkoxy having 1-8 carbons, amino, carboxy, sulfo and phenyl, wherein said phenyl is optionally further substituted by substituents selected from the group consisting of halogen, hydroxy, alkoxy having 1-8 carbons, aminoalkyl having 1-8 carbons, sulfoalkyl

Haugland and Yue  
Serial No. 09/557,275

and carboxyalkyl having 1-8 carbons; or one or more of  $R^{21}$ ,  $R^{22}$  and  $R^{23}$ , taken in combination with SPACER and  $R^{20}$  or SPACER alone forms a 5- or 6-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring, the heteroatoms selected from O, N or S; where  $\Psi^-$  is a compatible counterion;

or

CAP and CAP' are independently



4. (Amended) The compound according to Claim 2, wherein each  $R^2$  is independently ethyl or methyl, each X is independently O or S, each n is independently 0 or 1, and  $R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are each hydrogen.

5. (Amended) The compound according to Claim 2, wherein at least one  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  is a CYCLIC SUBSTITUENT selected from the group consisting of aryl, heteroaryl, and cycloalkyl having 3-10 carbons wherein said CYCLIC SUBSTITUENT is individually and optionally substituted by TAIL, halogen, amino, or an alkyl containing moiety comprising 1-6 carbons.

6. (Amended) The compound according to Claim 3, wherein said TAIL comprises

Haugland and Yue  
Serial No. 09/557,275

Sub B1  
a1  
LINK that is a single covalent bond or  $\text{NR}^{20}$  wherein  $\text{R}^{20}$  is hydrogen or an alkyl; SAPCER and SPACER' that are independently a linear alkyl having 1-8 carbons or a 6-membered carbon ring; CAP and CAP' that are individually  $-\text{NR}^{21}\text{R}^{22}$ , or  $-\text{N}^+\text{R}^{21}\text{R}^{22}\text{R}^{23}$   $\Psi$ , wherein  $\text{R}^{21}$ ,  $\text{R}^{22}$ , and  $\text{R}^{23}$  are independently hydrogen, alkyl or cycloalkyl.

7. (Amended) The compound according to Claim 6, wherein  $\text{R}^4$  is a TAIL or BRIDGE.

8. (Amended) The compound according to Claim 6, wherein  $\text{R}^5$  is a TAIL, a CYCLIC SUBSTITUENT, or BRIDGE.

9. (Amended) The compound according to Claim 8, wherein  $\text{R}^5$  is a TAIL or a BRIDGE wherein TAIL and BRIDGE incorporate at least one quaternary nitrogen atom.

10. (Amended) The compound according to Claim 2, wherein each  $\text{R}^3$ ,  $\text{R}^{11}$ ,  $\text{R}^{12}$ ,  $\text{R}^{13}$  and  $\text{R}^{14}$  is hydrogen.

11. (Amended) The compound according to Claim 3, wherein

$\text{R}^5$  is an alkyl and

$\text{R}^4$  is selected from the group consisting of halogen, CYCLIC SUBSTITUENT,  $-\text{OR}^8$ ,  $-\text{SR}^8$ ,  $-(\text{NR}^8\text{R}^9)$ , TAIL, BRIDGE,  $-\text{L-R}_x$ , and  $-\text{L-Sc}$ .

12. (Amended) The compound according to Claim 1, wherein  $\text{Sc}$  is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, an oligonucleotide, a nucleic acid, a lipid, a polymeric microparticle, a biological cell, a DNA-binding protein and a virus.

13. (Amended) The compound according to Claim 12, wherein  $\text{Sc}$  is an oligonucleotide, a nucleic acid, or a DNA-binding protein.



Haugland and Yue  
Serial No. 09/557,275

14. (Amended) The compound according to Claim 6, wherein

each X is O;

each n is independently = 0, 1, or 2;

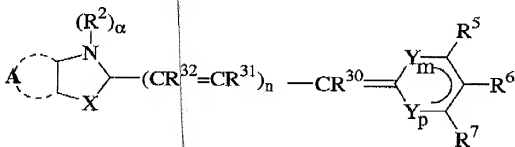
each m = 1;

each R<sup>30</sup>, R<sup>31</sup>, and R<sup>32</sup> are H; and

R<sup>5</sup> is selected from the group consisting of an alkyl, a TAIL, a CYCLIC SUBSTITUENT, and a BRIDGE.

15. (Amended) A complex comprising:

a) a compound having formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R<sup>2</sup>)<sup>β</sup>, provided at least one of said ring atoms is N(R<sup>2</sup>)<sup>β</sup> wherein aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl

Haugland and Yue  
Serial No. 09/557,275

having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio,  $-L-R_x$  and  $-L-S_c$ ;

X is selected from the group consisting of O, S, Se,  $NR^{15}$ , and  $CR^{16}R^{17}$  wherein  $R^{15}$  is hydrogen or an alkyl group having 1-6 carbons and  $R^{16}$  and  $R^{17}$  are independently alkyl groups having 1-6 carbons, or  $R^{16}$  and  $R^{17}$  taken in combination complete a five or six membered saturated ring;

$\alpha$  is 0 or 1 and  $\beta$  is 0 or 1 provided that  $\alpha + \beta = 1$ ;

$R^2$  is selected from the group consisting of  $-L-R_x$ ,  $-L-S_c$ , TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by sulfo, carboxy, or amino;

$n = 0, 1$  or  $2$ ;

Y is  $-CR^3=CR^4-$ ;

$p$  and  $m = 0$  or  $1$ , such that  $p + m = 1$ ;

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , TAIL, BRIDGE,  $-L-R_x$  and  $-L-S_c$  wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(CH_2)_2-W-(CH_2)_2-$  where W is a single bond,  $-O-$ ,  $-CH_2-$ , or  $-NR^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of hydrogen, halogen,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$  a CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$  and a saturated or unsaturated alkyl having 1-6 carbons that is linear or

Haugland and Yue  
Serial No. 09/557,275

Sub  
B1  
a1  
branched;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, -L- $R_6$ , -L- $S_6$ , a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of hydrogen alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$  is BRIDGE;

wherein

L and BRIDGE are independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

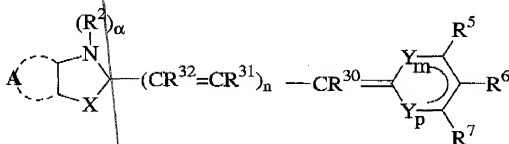
$R_x$  is a reactive group;

$S_6$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula

Haugland and Yue  
Serial No. 09/557,275



wherein A, X,  $R^2$ ,  $\alpha$ , n,  $Y_m$ ,  $Y_p$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{30}$ ,  $R^{31}$  and  $R^{32}$  are as defined above provided that BRIDGE not be any of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$ ; and

b) a nucleic acid polymer.

16. (Amended) The complex according to Claim 15, wherein said nucleic acid polymer is a chromosome or fragment thereof, or a natural or synthetic oligonucleotide.

17. (Cancelled)

18. (Amended) The complex according to Claim 16, wherein said nucleic acid is enclosed in a biological structure, free in solution, immobilized on a solid or semi-solid material or is extracted from a biological structure.

19. (Amended) The complex according to Claim 18, wherein said complex is enclosed in a biological structure present in a solution or on an inert matrix.

20. (Amended) The complex according to Claim 19 or 21, wherein said complex is enclosed in a biological structure.

21. (Amended) The complex according to Claim 18, wherein said biological structure is a cell and said cell is undergoing apoptosis, necrosis, or is in a cycle of cell division.

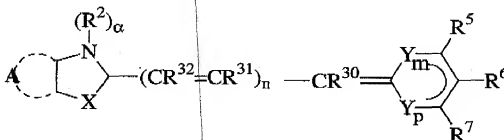
Haugland and Yue  
Serial No. 09/557,275

22. (Amended) The complex according to Claim 15, wherein at least one compound is substituted by  $-L-S_C$  wherein  $S_C$  is selected from the group consisting of hapten, nucleotide, oligonucleotide, nucleic acid polymer, protein, polysaccharide and DNA binding protein.

23. (Cancelled)

24. (Amended) A complex comprising:

a) a compound having formula:



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of  $-C-$ ,  $CH$  or  $N(R^2)^\beta$ , provided at least one of said ring atoms is  $N(R^2)^\beta$  wherein aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio,  $-L-R_x$  and  $-L-S_C$ ;

X is selected from the group consisting of O, S, Se,  $NR^{15}$ , and  $CR^{16}R^{17}$  wherein  $R^{15}$  is H or an alkyl group having 1-6 carbons and  $R^{16}$  and  $R^{17}$  are independently alkyl groups having 1-6 carbons, or  $R^{16}$  and  $R^{17}$  taken in combination complete a five or six membered

Haugland and Yue  
Serial No. 09/557,275

saturated ring

$\alpha$  is 0 or 1 and  $\beta$  is 0 or 1 provided that  $\alpha + \text{all } \beta = 1$ ;

$R^2$  is selected from the group consisting of  $-L-R_x$ ,  $-L-S_c$ , TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by sulfo, carboxy, or amino;

$n = 0, 1$  or  $2$ ;

Y is  $-\text{CR}^3=\text{CR}^4-$ ;

$p$  and  $m = 0$  or  $1$ , such that  $p + m = 1$ ;

$R^3$ ,  $R^4$ ,  $R^5$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-\text{OR}^8$ ,  $-\text{SR}^8$ ,  $-(\text{NR}^8\text{R}^9)$ , TAIL, BRIDGE,  $-L-R_x$  and  $-L-S_c$  wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(\text{CH}_2)_2\text{-W-(CH}_2)_2-$  where W is a single bond,  $-\text{O}-$ ,  $-\text{CH}_2-$ , or  $-\text{NR}^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of hydrogen, halogen,  $-\text{OR}^8$ ,  $-\text{SR}^8$ ,  $-(\text{NR}^8\text{R}^9)$  a CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$  and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$ , a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

Haugland and Yue  
Serial No. 09/557,275

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of hydrogen, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$  is BRIDGE;

wherein

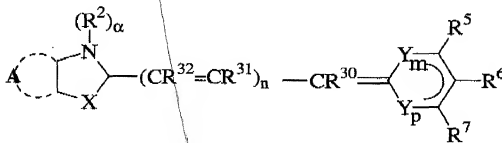
L and BRIDGE are independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

$R_x$  is a reactive group;

$S_c$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula



wherein A, X,  $R^2$ ,  $\alpha$ , n,  $Y_m$ ,  $Y_p$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{30}$ ,  $R^{31}$  and  $R^{32}$  are as defined above provided that BRIDGE not be any of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$ ; and,

Haugland and Yue  
Serial No. 09/557,275

b) a poly(amino acid).

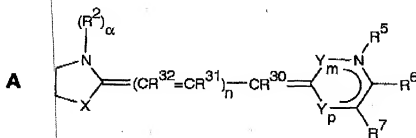
25. (Amended) The complex according to Claim 51, where said detergent is an alkyl sulfate or alkyl sulfonate salt.

26. (Amended) The complex according to Claim 25, wherein said poly(amino acids) are present on or in a solid or semi-solid matrix.

27. (Amended) The complex according to Claim 26, wherein said matrix is a membrane or an electrophoretic gel.

28. (Withdrawn) A method of staining poly(amino acids), comprising the steps of:

a) combining a sample that contains or is thought to contain a poly(amino acid) with a staining mixture that contains one or more dyes having the formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring, at least one of which is a nitrogen atom, said ring or rings being optionally further substituted one or more times by alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, or  $-\text{L}-\text{R}_x$ ; or  $-\text{L}-\text{S}_x$ ;



Haugland and Yue  
Serial No. 09/557,275

X is O, S, Se,  $\text{NR}^{15}$ , or  $\text{CR}^{16}\text{R}^{17}$ , where  $\text{R}^{15}$  is H or an alkyl group having 1-6 carbons; and  $\text{R}^{16}$  and  $\text{R}^{17}$ , which may be the same or different, are independently alkyl groups having 1-6 carbons, or  $\text{R}^{16}$  and  $\text{R}^{17}$  taken in combination complete a five or six membered saturated ring;

$\alpha$  is 0 or 1;

$\text{R}^2$  is an alkyl group having 1-6 carbons that is optionally substituted by sulfonate, carboxy, or amino; or  $\text{R}^2$  is  $-\text{L}-\text{R}_x$  or  $-\text{L}-\text{S}_x$ ; or TAIL; or BRIDGE-DYE;

$n = 0, 1$  or 2;

Y is  $-\text{CR}^3=\text{CR}^4-$ ;

$p$  and  $m = 0$  or 1, such that  $p + m = 1$ ;

$\text{R}^3, \text{R}^4, \text{R}^6$ , and  $\text{R}^7$  are independently H; an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or a halogen; or a CYCLIC SUBSTITUENT; or  $-\text{OR}^8$ ,  $-\text{SR}^8$ ,  $-(\text{NR}^8\text{R}^9)$ ; or TAIL; or BRIDGE-DYE; or  $-\text{L}-\text{R}_x$ ; or  $-\text{L}-\text{S}_x$ ; where  $\text{R}^8$  and  $\text{R}^9$ , which can be the same or different, are independently alkyl groups having 1-6 carbons; or 1-2 alicyclic or aromatic rings; or  $\text{R}^8$  and  $\text{R}^9$  taken in combination are  $-(\text{CH}_2)_2-\text{V}-(\text{CH}_2)_2-$  where V is a single bond,  $-\text{O}-$ ,  $-\text{CH}_2-$ , or  $-\text{NR}^{10}$ , where  $\text{R}^{10}$  is H or an alkyl having 1-6 carbons;

or  $\text{R}^6$  and  $\text{R}^7$  form a fused aromatic ring  $-\text{R}^{11}=\text{R}^{12}-\text{R}^{13}=\text{R}^{14}-$  wherein  $\text{R}^{11}, \text{R}^{12}, \text{R}^{13}$ , and  $\text{R}^{14}$  are optionally and independently alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or  $-\text{OR}^8$ ,  $-\text{SR}^8$ , or  $-(\text{NR}^8\text{R}^9)$ ; or a CYCLIC SUBSTITUENT; or a TAIL; or BRIDGE-DYE; or  $-\text{L}-\text{R}_x$ ; or  $-\text{L}-\text{S}_x$ ;

$\text{R}^5$  is an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or

Haugland and Yue  
Serial No. 09/557,275

$R^5$  is a CYCLIC SUBSTITUENT; or  $R^5$  is TAIL; or BRIDGE-DYE; or  $-L-R_x$ ; or  $-L-S_c$ ;  
or  $R^5$  is a pair of electrons;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently H,  $C_1-C_6$  alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl or heteroaryl;

wherein

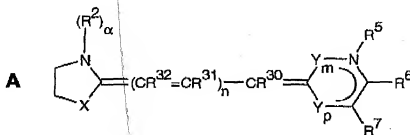
L and BRIDGE are independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-16 nonhydrogen atoms selected from the group consisting of C, N, P, O and S, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds;

$R_x$  is a reactive group;

$S_c$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound of the formula



Haugland and Yue  
Serial No. 09/557,275

wherein A, X, R<sup>2</sup>,  $\alpha$ , n, Y<sub>m</sub>, Y<sub>p</sub>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>,  
R<sup>15</sup>, R<sup>16</sup>, R<sup>17</sup>, R<sup>18</sup>, R<sup>19</sup>, R<sup>20</sup>, R<sup>21</sup>, R<sup>22</sup>, R<sup>23</sup>, R<sup>24</sup>, R<sup>30</sup>, R<sup>31</sup>, R<sup>32</sup>, TAIL, CYCLIC  
SUBSTITUENT are as defined above;

that is bound to BRIDGE at one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, or R<sup>7</sup>;

b) incubating the combined mixture for a time sufficient for the dye in the staining mixture to associate with the poly(amino acid) in the sample mixture to form a dye-poly(amino acid) complex that gives a detectable optical response upon illumination;

d) illuminating said dye-poly(amino acid) complex; and

e) observing said detectable optical response.

29. (Withdrawn) A method, as claimed in Claim 28, further comprising heating the sample mixture prior to combining with the staining mixture, or heating the combined mixture.

30. (Withdrawn) A method, as claimed in Claim 28, further comprising removing, destroying, or dispersing below the critical micelle concentration any biological membranes that are present in the sample mixture.

31. (Withdrawn) A method, as claimed in Claim 28, further comprising adding an anionic detergent to the sample mixture, staining mixture or combined mixture.

Haugland and Yue  
Serial No. 09/557,275

32. (Withdrawn) A method, as claimed in Claim 31, wherein said detergent is an alkyl sulfate or alkyl sulfonate salt having 6-18 carbons; that is present in a concentration of less than 0.1% by weight.

33. (Withdrawn) A method, as claimed in Claim 28, wherein said detectable optical response is a colorimetric response.

34. (Withdrawn) A method, as claimed in Claim 28, wherein said detectable optical response is a fluorescence response.

35. (Withdrawn) A method, as claimed in Claim 28, further comprising quantitating said poly(amino acid) by measuring said detectable optical response and comparing said measurement with a standard.

36. (Withdrawn) A method, as claimed in Claim 28, further comprising electrophoretically separating the sample mixture before, after, or while it is combined with the staining mixture.

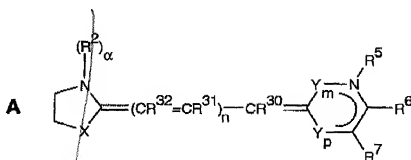
37. (Withdrawn) A method, as claimed in Claim 28, further comprising transferring the sample mixture to a solid or semi-solid matrix before or after combining with the staining mixture.

38. (Withdrawn) A method, as claimed in Claim 28, further comprising adding an additional reagent to the sample mixture, the staining mixture, or the combined mixture.

39. (Withdrawn) A method of staining nucleic acids, comprising

a) combining a sample that contains or is thought to contain a nucleic acid with a mixture containing a dye compound of the formula

Haugland and Yue  
Serial No. 09/557,275



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring, at least one of which is a nitrogen atom, said ring or rings being optionally further substituted one or more times by alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, or -L-R<sub>x</sub>; or -L-S<sub>c</sub>;

X is O, S, Se, NR<sup>15</sup>, or CR<sup>16</sup>R<sup>17</sup>, where R<sup>15</sup> is H or an alkyl group having 1-6 carbons; and R<sup>16</sup> and R<sup>17</sup>, which may be the same or different, are independently alkyl groups having 1-6 carbons, or R<sup>16</sup> and R<sup>17</sup> taken in combination complete a five or six membered saturated ring;

$\alpha$  is 0 or 1;

R<sup>2</sup> is an alkyl group having 1-6 carbons that is optionally substituted by sulfonate, carboxy, or amino; or R<sup>2</sup> is -L-R<sub>x</sub> or -L-S<sub>c</sub>; or TAIL; or BRIDGE-DYE;

$n = 0, 1$  or  $2$ ;

Y is -CR<sup>3</sup>=CR<sup>4</sup>-;

$p$  and  $m = 0$  or  $1$ , such that  $p + m = 1$ ;

Haugland and Yue  
Serial No. 09/557,275

Sub  
B1

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently H; an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or a halogen; or a CYCLIC SUBSTITUENT; or  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ ; or TAIL; or BRIDGE-DYE; or  $-L-R_x$ ; or  $-L-S_x$ ; where  $R^8$  and  $R^9$ , which can be the same or different, are independently alkyl groups having 1-6 carbons; or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(CH_2)_2-V-(CH_2)_2-$  where V is a single bond,  $-O-$ ,  $-CH_2-$ , or  $-NR^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

ai

or  $R^6$  and  $R^7$  form a fused aromatic ring  $-R^{11}=R^{12}-R^{13}=R^{14}-$  wherein  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are optionally and independently alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or  $-OR^8$ ,  $-SR^8$ , or  $-(NR^8R^9)$ ; or a CYCLIC SUBSTITUENT; or a TAIL; or BRIDGE-DYE; or  $-L-R_x$ ; or  $-L-S_x$ ;

$R^5$  is an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or  $R^5$  is a CYCLIC SUBSTITUENT; or  $R^5$  is TAIL; or BRIDGE-DYE; or  $-L-R_x$ ; or  $-L-S_x$ ; or  $R^5$  is a pair of electrons;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently H,  $C_1$ - $C_6$  alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, or heteroaryl;

wherein

L and BRIDGE are independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-16 nonhydrogen atoms selected from the group consisting of C, N, P, O and S, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds;

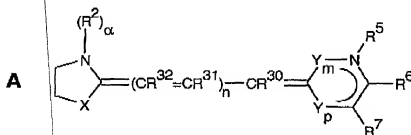
Haugland and Yue  
Serial No. 09/557,275

$R_x$  is a reactive group;

$S_c$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound of the formula



wherein A, X,  $R^2$ ,  $\alpha$ , n,  $Y_m$ ,  $Y_p$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$ ,  $R^{16}$ ,  $R^{17}$ ,  $R^{18}$ ,  $R^{19}$ ,  $R^{20}$ ,  $R^{21}$ ,  $R^{22}$ ,  $R^{23}$ ,  $R^{24}$ ,  $R^{30}$ ,  $R^{31}$ ,  $R^{32}$ , TAIL, CYCLIC SUBSTITUENT are as defined above;

that is bound to BRIDGE at one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ , or  $R^7$ ;

b) incubating the sample and mixture for a time sufficient for the dye compound to combine with the nucleic acid in the sample to form one or more dye-nucleic acid complexes that give a detectable fluorescent signal.

40. (Withdrawn) A method of staining nucleic acids, as claimed in Claim 39, wherein said sample or said mixture comprises an electrophoretic gel.

41. (Withdrawn) A method of staining nucleic acids, as claimed in Claim 39, wherein the

Haugland and Yne  
Serial No. 09/557,275

sample comprises a biological fluid.

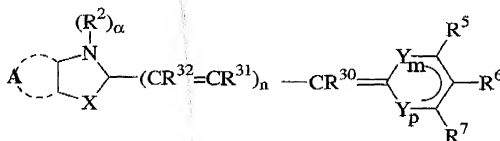
42. (Withdrawn) A method of staining nucleic acids, as claimed in Claim 39, wherein the sample comprises cells.

43. (Withdrawn) A method of staining nucleic acids, according to Claim 39, where the sample comprises cell-free nucleic acids.

44. (Amended) A kit comprising:

a) a stock solution comprising:

i) one or more compounds individually having formula:



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R<sup>2</sup>)<sup>β</sup>, provided at least one of said ring atoms is N(R<sup>2</sup>)<sup>β</sup> wherein aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, -L-R<sub>x</sub> and -L-S<sub>x</sub>;

X is selected from the group consisting of O, S, Se, NR<sup>15</sup>, and CR<sup>16</sup>R<sup>17</sup> wherein R<sup>15</sup> is hydrogen or an alkyl group having 1-6 carbons and R<sup>16</sup> and R<sup>17</sup> are independently alkyl groups having 1-6 carbons, or R<sup>16</sup> and R<sup>17</sup> taken in combination complete a five or six



Haugland and Yue  
Serial No. 09/557,275

membered saturated ring;

$\alpha$  is 0 or 1 and  $\beta$  is 0 or 1 provided that  $\alpha + \text{all } \beta = 1$ ;

$R^2$  is selected from the group consisting of  $-L-R_x$ ,  $-L-S_c$ , TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by sulfo, carboxy, or amino;

$n = 0, 1$  or  $2$ ;

$Y$  is  $-\text{CR}^3=\text{CR}^4-$ ;

$p$  and  $m = 0$  or  $1$ , such that  $p + m = 1$ ;

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-\text{OR}^8$ ,  $-\text{SR}^8$ ,  $-(\text{NR}^8\text{R}^9)$ , TAIL, BRIDGE,  $-L-R_x$  and  $-L-S_c$  wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(\text{CH}_2)_2\text{-W-(CH}_2)_2-$  where  $W$  is a single bond,  $-\text{O-}$ ,  $-\text{CH}_2-$ , or  $-\text{NR}^{10}$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of hydrogen, halogen,  $-\text{OR}^8$ ,  $-\text{SR}^8$ ,  $-(\text{NR}^8\text{R}^9)$  a CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$  and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_c$ , a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

Haugland and Yue  
Serial No. 09/557,275

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of H, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$  is BRIDGE;

wherein

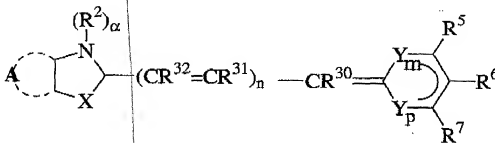
L and BRIDGE are independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

$R_x$  is a reactive group;

$S_c$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula



wherein A, X,  $R^2$ ,  $\alpha$ , n,  $Y_m$ ,  $Y_p$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{30}$ ,  $R^{31}$  and  $R^{32}$  are as defined above provided that BRIDGE not be any of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$ ;

Haugland and Yue  
Serial No. 09/557,275

li) an organic solvent; and,

b) a buffer suitable for dilution of said stock solution.

45. (Cancelled)

46. (Amended) The kit according to Claim 44, further comprising a standard, an additional detection reagent, a silicon chip, a glass slide, or any combination thereof.

47. (Amended) The kit according to Claim 46, wherein said additional detection reagent is selected from the group consisting of an organelle stain, a labeled immunoreagent, a drug, and an enzyme.

48. (New) The kit according to Claim 47, wherein said R<sup>5</sup> is BRIDGE that is attached to said DYE.

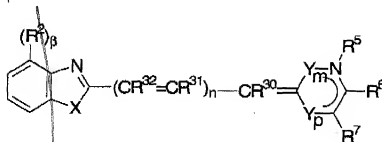
49. (New) The kit according to Claim 47, wherein said kit comprises two to six individual said compounds.

50. (New) The kit according to any one of claims Claim 47, 48 or 49, wherein said organic solvent is DMSO.

51. (New) The complex according to Claim 24, wherein said complex further comprises a detergent.

52. (New) A compound having formula:

Haugland and Yue  
Serial No. 09/557,275



wherein  $R^2$  is an alkyl having 1-6 carbon atoms and wherein meta and ortho positions to  $R^2$  are independently and optionally substituted with halogen or an alkyl having 1-6 carbon atoms;

X is selected from the group consisting of O, S, Se,  $NR^{15}$ , and  $CR^{16}R^{17}$  wherein  $R^{15}$  is H or an alkyl group having 1-6 carbons and  $R^{16}$  and  $R^{17}$  are independently alkyl groups having 1-6 carbons;

$n = 0, 1$  or  $2$ ;

$\beta = 1$ ;

Y is  $-CR^3=CR^4-$ ;

p and m = 0 or 1, such that  $p + m = 1$ ;

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , TAIL and BRIDGE wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(CH_2)_2-W-(CH_2)_2-$  where W is a single bond,  $-O-$ ,  $-CH_2-$ , or  $-NR^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of

Haugland and Yue  
Serial No. 09/557,275

hydrogen, halogen,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , CYCLIC SUBSTITUENT, TAIL, BRIDGE, and an alkyl having 1-6 carbons;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, sulfoalkyl and an alkyl having 1-6 carbons;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of H, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

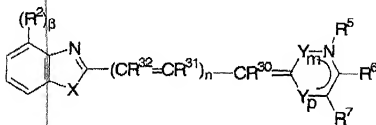
when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  is BRIDGE;

wherein;

BRIDGE is independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

TAIL is a heteroatom-containing moiety;

DYE is a compound of the formula



wherein X,  $R^2$ , n,  $Y_m$ ,  $Y_p$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{30}$ ,  $R^{31}$  and  $R^{32}$  are as defined above provided that BRIDGE not be any of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$ .

Haugland and Yue  
Serial No. 09/557,275

53. (New) The compound according to Claim 52, wherein

$R^2$  is methyl; said meta and ortho positions are optionally substituted by halogen; X is S or O;  $n = 0$ ;  $m = 1$ ;

$R^3$  and  $R^4$  are independently selected from the group consisting of H, alkyl, CYCLIC SUBSTITUENT and TAIL;

$R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently hydrogen,  $-OR^8$ , or an alkyl having 1-6 carbons wherein  $R^8$  is methyl;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, and methyl;

$R^{30}$  is hydrogen.

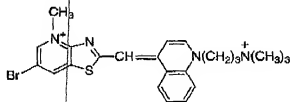
54. (New) The compound according to Claim 53, wherein X is S, said halogen is chlorine or bromine,  $R^3$  is hydrogen,  $R^4$  is hydrogen or an alkyl and  $R^5$  is selected from the group consisting of methyl, CYCLIC SUBSTITUENT, BRIDGE and TAIL wherein said CYCLIC SUBSTITUENT is an unsubstituted aryl and TAIL comprises formula -  $(CH_2)_3N(CH_3)_3$ .

55. (New) The compound according to Claim 54 or 53, wherein said  $R^5$  is BRIDGE comprising formula  $-(CH_2)_3N(CH_3)CH_2-$ .

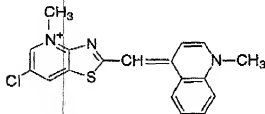
56. (New) The compound according to any one of Claims 55, 54 or 53, wherein said BRIDGE is attached to said DYE.

Haugland and Yue  
Serial No. 09/557,275

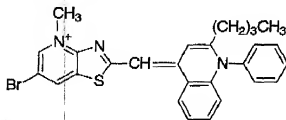
57. (New) The compound according to Claim 54, wherein said compound has the formula:



58. (New) The compound according to Claim 54, wherein said compound has the formula:



59. (New) The compound according to Claim 54, wherein said compound has the formula:

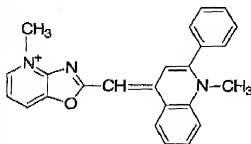


60. (New) The compound according to Claim 53, wherein X is O; R<sup>3</sup> is hydrogen; R<sup>4</sup> is selected from the group consisting of hydrogen, CYCLIC SUBSTITUENT, TAIL and an

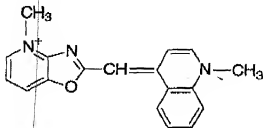
Haugland and Yue  
Serial No. 09/557,275

alkyl;  $R^5$  is selected from the group consisting of methyl, CYCLIC SUBSTITUENT, BRIDGE and TAIL wherein said CYCLIC SUBSTITUENT is an unsubstituted aryl and said TAIL comprises LINK that is a single covalent bond, SPACER that is a phenyl ring and CAP comprising formula  $-(CH)_2N^+CH_3(CH_2CH_3)_2$ ;  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are individually hydrogen or  $-OCH_3$ .

61. (New) The compound according to Claim 60, wherein said compound has the formula:



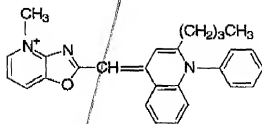
62. (New) The compound according to Claim 60, wherein said compound has the formula:



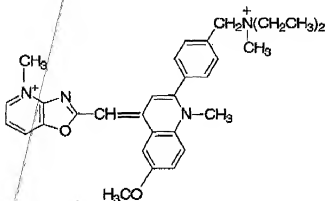
63. (New) The compound according to Claim 60, wherein said compound has the formula:



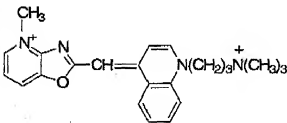
Haugland and Yue  
Serial No. 09/557,275



64. (New) The compound according to Claim 60, wherein said compound has the formula:

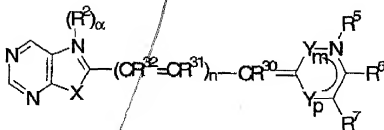


65. (New) The compound according to Claim 60, wherein said compound has the formula:



66. (New) A compound having formula:

Haugland and Yue  
Serial No. 09/557,275



wherein  $R^2$  is an alkyl having 1-6 carbon atoms and fused 6-membered aromatic ring is optionally substituted at a ring carbon by methylthio;

a  
X is selected from the group consisting of O, S, Se,  $NR^{15}$ , and  $CR^{16}R^{17}$  wherein  $R^{15}$  is hydrogen or an alkyl group having 1-6 carbons and  $R^{16}$  and  $R^{17}$  are independently alkyl groups having 1-6 carbons;

$n = 0, 1$  or  $2$ ;

$\alpha = 1$ ;

Y is  $-CR^3=CR^4$ ;

p and m = 0 or 1, such that  $p + m = 1$ ;

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , TAIL and BRIDGE wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(CH_2)_2-W-(CH_2)_2$  where W is a single bond,  $-O-$ ,  $-CH_2-$ , or  $-NR^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of hydrogen, halogen,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , CYCLIC SUBSTITUENT, TAIL, BRIDGE,

Haugland and Yue  
Serial No. 09/557,275

and an alkyl having 1-6 carbons;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, sulfoalkyl and an alkyl having 1-6 carbons;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of H, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

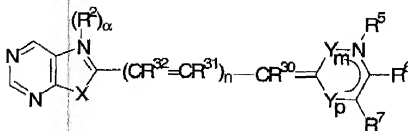
when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  is BRIDGE;

wherein;

BRIDGE is independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula

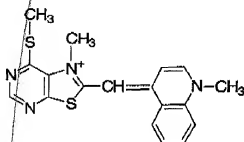


wherein X,  $R^2$ , n,  $Y_m$ ,  $Y_p$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{30}$ ,  $R^{31}$  and  $R^{32}$  are as defined above provided that BRIDGE not be any of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$ .

67. (New) The compound according to Claim 66, wherein said 6-membered aromatic ring is substituted by methylthio; X is S; n is 0; m is 1;  $R^3$ ,  $R^4$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$  and  $R^{14}$  are hydrogen and  $R^5$  is methyl.

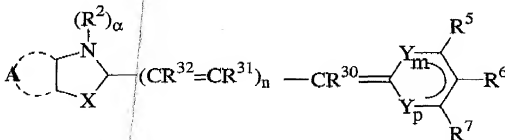
Haugland and Yue  
Serial No. 09/557,275

68. (New) The compound according to Claim 67, wherein said compound has the formula:



69. (New) A solution for staining nucleic acid polymers or poly(amino acids) wherein said solution comprises:

a) one or more compounds having formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of  $-C-$ ,  $CH$  or  $N(R^2)^\beta$ , provided at least one of said ring atoms is  $N(R^2)^\beta$  wherein aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio,  $-L-R_x$  and  $-L-S_x$ ;

X is selected from the group consisting of O, S, Se,  $NR^{15}$ , and  $CR^{16}R^{17}$  wherein  $R^{15}$  is

Haugland and Yue  
Serial No. 09/557,275

hydrogen or an alkyl group having 1-6 carbons and  $R^{16}$  and  $R^{17}$  are independently alkyl groups having 1-6 carbons, or  $R^{16}$  and  $R^{17}$  taken in combination complete a five or six membered saturated ring;

$\alpha$  is 0 or 1 and  $\beta$  is 0 or 1 provided that  $\alpha + \beta = 1$ ;

$R^2$  is selected from the group consisting of  $-L-R_x$ ,  $-L-S_x$ , TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by sulfo, carboxy, or amino;

$n = 0, 1$  or  $2$ ;

$Y$  is  $-CR^3=CR^4-$ ;

$p$  and  $m = 0$  or  $1$ , such that  $p + m = 1$ ;

$R^3$ ,  $R^4$ ,  $R^6$ , and  $R^7$  are independently selected from the group consisting of hydrogen, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , TAIL, BRIDGE,  $-L-R_x$  and  $-L-S_x$  wherein  $R^8$  and  $R^9$  are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or  $R^8$  and  $R^9$  taken in combination are  $-(CH_2)_2-W-(CH_2)_2-$  where  $W$  is a single bond,  $-O-$ ,  $-CH_2-$ , or  $-NR^{10}-$ , where  $R^{10}$  is H or an alkyl having 1-6 carbons;

or  $R^6$  and  $R^7$  taken in combination form a fused 6-membered aromatic ring wherein ring substituents  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ , and  $R^{14}$  are independently selected from the group consisting of hydrogen, halogen,  $-OR^8$ ,  $-SR^8$ ,  $-(NR^8R^9)$ , a CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_x$  and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

$R^5$  is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE,  $-L-R_x$ ,  $-L-S_x$ , a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-

Haugland and Yee  
Serial No. 09/557,275

6 carbons that is linear or branched;

$R^{30}$ ,  $R^{31}$ , and  $R^{32}$  are independently selected from the group consisting of hydrogen, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ ,  $R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$  and  $R^{15}$  is BRIDGE;

wherein

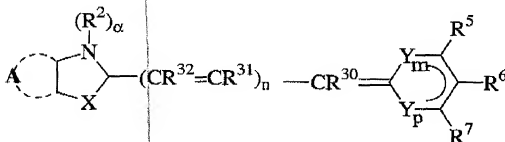
L and BRIDGE are independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

$R_x$  is a reactive group;

$S_c$  is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula

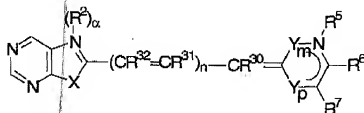


Haugland and Yue  
Serial No. 09/557,275

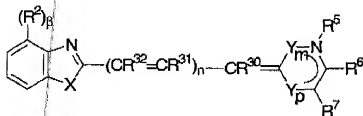
wherein A, X, R<sup>2</sup>,  $\alpha$ , n, Y<sub>m</sub>, Y<sub>p</sub>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>30</sup>, R<sup>31</sup> and R<sup>32</sup> are as defined above provided that BRIDGE not be any of R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup> and R<sup>15</sup>; and,

b) an organic solvent.

70. (New) The solution according to Claim 69, wherein said compound has the formula



or



71. (New) The solution according to Claim 70, wherein R<sup>2</sup> is methyl; said 6-membered aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, halogen, methylthio; X is S or O; n = 0; m = 1;

R<sup>3</sup> and R<sup>4</sup> are independently selected from the group consisting of hydrogen, alkyl, CYCLIC SUBSTITUENT and TAIL;

Haugland and Yue  
Serial No. 09/557,275

*sub*  
*89*  
R<sup>6</sup> and R<sup>7</sup> taken in combination form a fused 6-membered aromatic ring wherein ring substituents R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, and R<sup>14</sup> are independently hydrogen, -OR<sup>9</sup>, or an alkyl having 1-6 carbons wherein R<sup>8</sup> is methyl;

*all*  
*correct*  
R<sup>5</sup> is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, and methyl;

R<sup>30</sup> is hydrogen.

72. (New) The solution according to Claim 71, wherein R<sup>3</sup> is hydrogen, said CYCLIC SUBSTITUENT is an unsubstituted aryl and TAIL comprises LINK that is a single covalent bond, SPACER that is a phenyl ring or a linear alkyl and CAP comprising formula  $-(CH_2)_n-CH_3$  (CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub> or formula  $-N(CH_3)_3$ .

73. (New) The solution according to Claim 72, wherein R<sup>5</sup> is BRIDGE comprising formula  $-(CH_2)_3N(CH_3)CH_2-$  wherein DYE is attached to said BRIDGE.

74. (New) The solution according to Claim 72 or 73 wherein said organic solvent is DMSO.



Haugland and Yue  
Serial No. 09/557,275

Respectfully submitted,

Date: Aug. 21, 2002

*Koren J. Anderson*

Koren J. Anderson, PhD  
Reg. No. 51,061

Molecular Probes, Inc.  
P.O. Box 22010  
Eugene, Oregon, 97402  
Phone: (541) 984-5656  
Facsimile: (541) 465- 8354